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Calcium Carbonate Reinforced Natural Polymer Composite For Bone Grafts

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ABSTRACT

Challenges in tissue engineering have always-motivated scientists and engineers to develop new biomaterials that can restore the structural features and physiological functions of natural tissues. A novel ceramic-polymer composite was processed with bio-active ceramics dispersed in a natural bio-active polymer for bone graft applications. A commercially available caster bean extract polymer (CBP) was used. It is a natural polymer extracted from the oily caster beans of the dicotyledonous class. During processing of these composites, *in situ* random interconnected porosity was generated similar to natural bone. Hg-porosimetry results of these composites show that most of the pores are between 50 to 150 microns. Compression tests were performed on cylindrical samples to determine the mechanical properties. Average compression modulus was calculated as 173 MPa, while the average failure strength was 6.7 MPa. Cytotoxicity and cell proliferation studies were conducted with modified human osteoblast cell-line (OPC-1) to show that these composites are biocompatible. Composites showed good cell attachment with a continuous increase in cell growth for at least up to two weeks.

INTRODUCTION

During the past four decades, applications of novel materials for biomedical practices have greatly revolutionized the quality of human life. Many speciality polymers, metals, ceramics and composites have been developed for numerous applications [1]. These materials in various forms and phases are made to perform different functions in repair and reconstruction of diseased and damaged parts of the human body. Among all materials, the ceramics, and more precisely porous ceramics, are the most suitable ones for bone graft applications, primarily because bone itself is a naturally occurring porous ceramic. One of the restrictions on clinical uses of ceramics is the uncertain lifetime under the complex stress states due to poor resistance to crack propagation. Introduction of porosity increases these uncertainties to a significantly higher level. This concern has resulted in limited applications of porous ceramics.

Significant research has already taken place to develop polymer-ceramic composites as biomaterials. These composites attempt to incorporate the advantages of both the ceramic and the polymer phases. Most of the researches on polymer ceramic composites have been focused on (a) bio-inert polymer with bio-active ceramics [2, 3], and (b) bio-active polymer with bio-active ceramics [4, 5]. In our research, a novel ceramic-polymer composite was processed with bio-active ceramics dispersed in a natural bio-active polymer. Moreover, during processing of these composites, *in situ* random interconnected porosity was generated, which are similar to

natural bone. The composite material was characterized for their physical, mechanical and biological properties.

EXPERIMANTAL PROCEDURE

A commercially available natural polymer was used for this study. The commercial name is "Polimero Vegetal Osteointegraval". Vegetable polymer was extracted from the "Ricinus Comunis" (mamona oil), oil plant belonging to the dicotiledônea class, gerianaceas order and euforbaceas family, its recinoleic acid structure cointain 18 carbon atoms in which there is a double bond between the 9^{th} and the 10^{th} carbons. Calcium carbonate powder was mixed with this polymer to form a ceramic-polymer composite.

The natural polymer comes in two parts. These two parts are named as "polylol" and "pre-polymero". During processing of this composite, calcium carbonate powder was added to the liquid polymer while mixing the two components prior to room temperature curing. Preparation was carried out in a clean glass beaker. Mixing was done manually. A total of 6 grams $CaCO_3$ was added to 40 ml of polymer mixture during polymerization. After 1 minute of mixing, there was an increase in material temperature to 45^0C. The total curing time was between 3 to 5 minutes. The polymer was taken out of the beaker and allowed to cure in another clean glass beaker. During polymerization, *in situ* random interconnected pores were generated. After curing, the overall material resulted into a strong porous composite with three-dimensionally interconnected porosity.

Cylindrical samples were core drilled with uniform diameter of 11.5 mm. These samples were then sectioned to a length of 20 mm each to test under uniaxial compression loading. For *in vitro* testing, circular disk samples were prepared. *In vitro* samples had an average diameter of 11.5 mm and an average thickness of 1.8 mm. Biodegradation studies were carried out with *in vitro* samples in culture media. Hg-porosimetry was carried out to evaluate the pore size and their distribution.

In vitro testing was carried out using a human osteoblast cell-line (OPC 1). The OPC-1 cell line is a conditionally immortalized osteoprecursor cell line derived from human fetal bone tissue [6]. The OPC 1 cells were cultured in a standard medium made of Mccoy's 5A (with l-Glutamine, without Phenol Red and Sodium Bicarbonate) [Sigma Chemical Co, Saint Louis, MO], supplemented with 10% fetal bovine serum, 2.2 gram/liter sodium bicarbonate, 0.1 gram/liter penicillin and 0.1 gram/liter streptomycin. The selection of a nutrient medium is strongly influenced by type of cell, type of culture and degree of necessary chemical definition. Cells were removed and split in the ratio 1:2, 3 days before use. The cells were seeded on to 15 disk samples. The cultures were incubated at 37 ^{0}C in a humidified 5% CO_2 atmosphere. Cell counts were taken after 3, 7, 14 and 21 days of culture. Microscopic observations revealed that the cells had attached, anchored and spread on to the porous disk samples within 3 days after seeding. A colorimetric assay (MTT assay) was used to evaluate living cell number at various times after seeding. This assay quantitates the ability of mitochondrial dehydrogenases to metabolize 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide to an insoluble formazan [7]. The amount of formazan is directly proportional to the total number of living cells. This enzyme assay produces a colored product, which is quantitated in a microplate reader at a wavelength of 570 nm.

RESULTS AND DISCUSSION

Bulk density and mercury porosimetry

Bulk density of the composite was determined using cylindrical samples. The bulk density was found to be 0.4 g/cm^3. The composite porosity was determined using a Hg-porosimeter (Auto Pore 9400, Micromeretics, GA) and a typical result is shown in figure 1. Hg- porosimetry is a useful method for determining pore size and distribution in porous structures of many materials. It can be seen from the plot that the majority of the pores were in the range of 50-100 μm, while some were between 200-250 μm. The porosimetry result also indicates that the bulk density of the sample was 0.4 g/cm^3 with an apparent density of 0.51 g/cm^3. The composite samples had an average total porosity of 24 vol %. Figure 2 shows an SEM micrograph of the composite. The typical pore sizes and their interconnectivity can be seen from this figure.

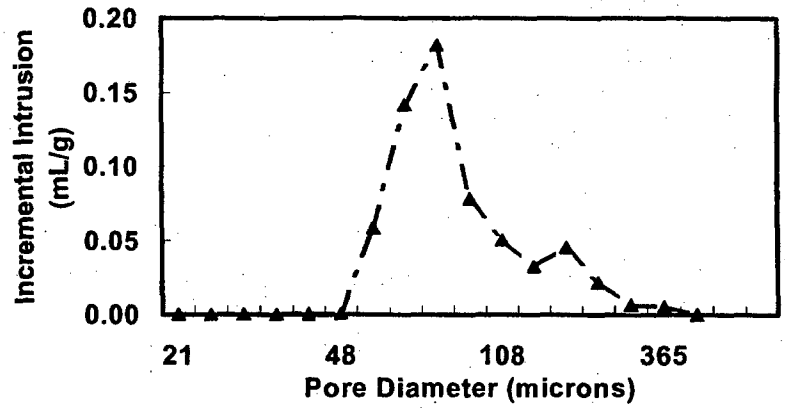

Figure 1. Hg porosimetry plot shows the variation of pore sizes in the composite.

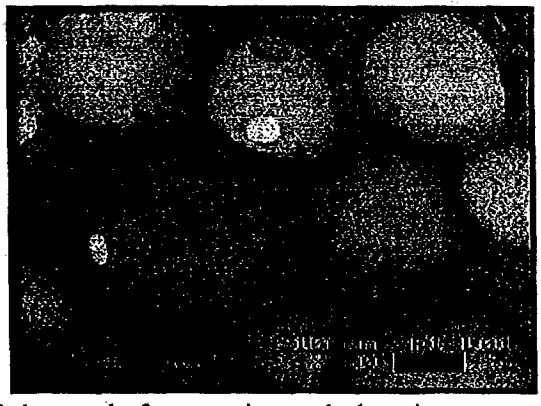

Figure 2. An SEM photograph of a composite sample shows interconnected spherical open pores in the size range of 50-100 microns.

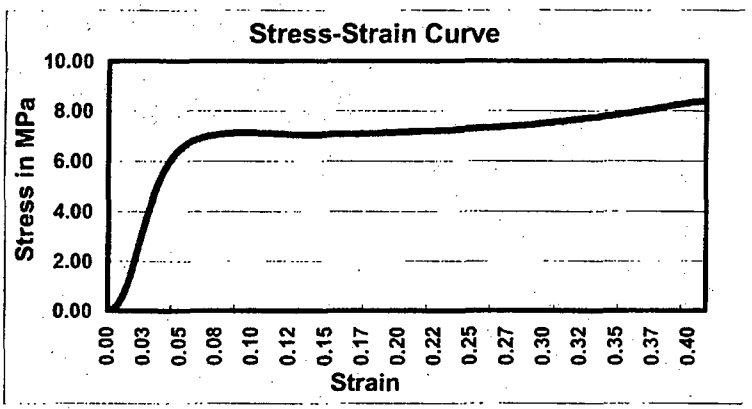

Figure 3. The stress-strain relationship of one of the compression samples

Mechanical testing

Uniaxial compression tests were performed on four samples of this composite to evaluate their mechanical properties. Figure 3 shows the stress-strain plot for one of the samples under compression testing. The modulus of compressibility and the stress at which failure initiates were determined from the stress-strain curves. Average compression modulus was calculated as 173 Mpa (+/- 16 MPa), while the average failure strength was 6.7 MPa (+/- 0.64 MPa). The failure strength is quite comparable to human cancellous bone, which has a typical compressive strength between 0.5-14.6 MPa [8].

***In vitro* Testing**

Biocompatibility is defined as the ability of a material to perform with an appropriate host response in a specific application. *In vitro* testing was done to test the biocompatibility of this composite. All the matrices were found to be non-toxic and biocompatible. Observations on the 3rd and 7th days of culture showed that the cell attachment and growth were very good. The cells were well spread and were in contact with each other. A continuous increase in cell growth was also observed up to 14th day. Cell counts on day 21st had indicated that cells had begun to detach from the matrices. Similar tests were repeated twice and the results were identical, which suggests that there was a saturation effect of cell growth in these matrices. A possible explanation for this growth behavior for cells during the third week could be due to lack of space for cells to grow. The matrix surfaces were saturated with cells after 14 days and then the cells started impinging to each other. Figure 4 shows the growth curve of OPC 1 cells on composite matrices as a function of time in days. Figure 5 shows the cell attachment on these matrices after 7th day.

Biodegradation studies were carried out on the composite using the same standard media that was used for *in vitro* testing. Fifteen samples were prepared and their dry weights were

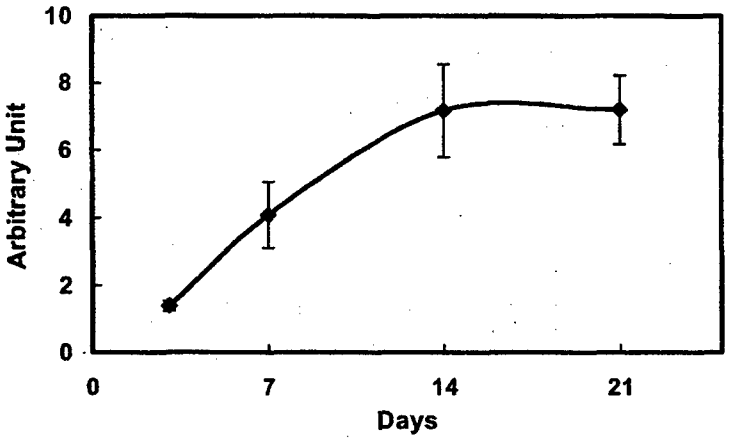

Figure 4. Growth curve of OPC 1 cells on polymer-ceramic composite matrices

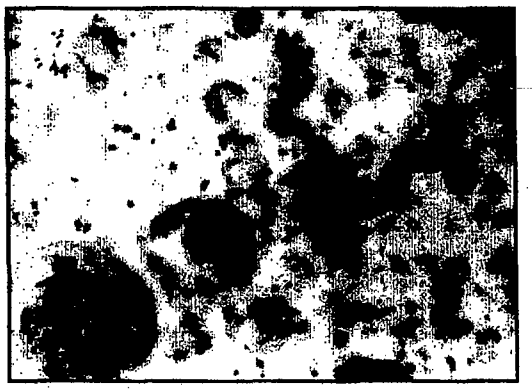

Figure 5. Cell attachment on the composite matrices on Day 7th

recorded. These samples were then immersed in the standard media and maintained at 37 °C in the incubator. Rate of dissolution was measured in terms of normalized dry weight loss. A negligible weight loss of around 1.5 weight% was recorded at the end of 28 days. The results indicate that the composite is bioresorbable, though the degradation rate is very slow.

CONCLUSIONS

Porous calcium carbonate reinforced natural polymer composite has been processed and characterized. Uniaxial compression tests showed the average strength of 6.7 MPa and the

compression modulus of 173 MPa. Hg-porosimetry revealed that the majority of the pores are in the range of 50-100 μm. *In vitro* testing with the OPC 1 cell-line showed that the composites are biocompatible. Biodegradation studies with Mccoy's 5A media showed that the composite is bioresorbable as well with a fairly slow degradation rate. The results are promising for these composites to be used as bone-graft materials, though further *in vivo* tests are necessary on these materials to be used successfully in various orthopedic applications.

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